The lectin nature of α-galactosidases from *Vicia faba* seeds

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Received 29 October 1982

α-Galactosidase from *Vicia faba* seeds has been resolved into three molecular forms, I, II^1 and II^2, respectively. Enzyme I is a tetramer (M_r 160000) consisting of identical sub-units (M_r 44000 ± 2000). All three forms display lectin activity with glucose/mannose specificity. Enzyme I has been further studied with respect to its lectin specificity and various factors affecting this property. The results indicate that the catalytic and the lectin sites reside in the same protein molecule. The results presented are unique in that the enzyme activity is specific for galactose and its lectin activity is specific for glucose/mannose.

Vicia faba *α-Galactosidase* *Lectin* *Multimolecular form*

1. INTRODUCTION

Carbohydrate-binding proteins which agglutinate erythrocytes and precipitate glycoconjugates are very common in plant tissues. These phytohaemagglutinins or lectins may be simple proteins or glycoproteins and they exhibit considerable binding specificity towards carbohydrates. The nature and properties of plant lectins have been reviewed in [1,2]. Leguminous seeds are a rich source of lectins many of which possess homologous segments of amino acids and, hence, may have an evolutionary relationship [3–5].

Few examples of enzymes possessing lectin activity have been reported but α-mannosidase from *Phaseolus vulgaris* seeds [6] and α-galactosidases obtained from several species of leguminous seeds [7] have been shown to be lectins.

α-Galactosidases from *Vigna radiata* (mung bean) seeds possess monomeric and tetrameric forms of the enzyme (M_r 40000–45000 and 160000, respectively) [8,9]. The tetramer is a lectin possessing D-galactose specificity.

2. METHODS

α-Galactosidase activity was assayed using *p*-nitrophenyl α-D-galactoside (PNPG) as substrate according to [11].

Lectin activity was assayed using a 1.5% suspension of rabbit blood erythrocytes, as in [12,13]. The sugar specificity of the lectin was analysed by observing 50% inhibition of agglutination on a visual serological scale [13].

Protein was determined as in [14] using bovine serum albumin as a standard.

This paper is mainly concerned with a study of α-galactosidase I from *Vicia faba* seeds, a tetrameric glycoprotein with glucose/mannose lectin specificity. This enzyme occurs together with its monomer, II^2, and a second low M_r enzyme, II^1, in resting seeds [10].

3. RESULTS AND DISCUSSION

The procedure used for the purification of the three forms of α-galactosidase from resting *Vicia faba* seeds was essentially similar to that in [10], although the con A–Sepharose stage was omitted to avoid possible contamination with concanavalin A. In addition, enzymes I and II were recycled twice through a Sephadex G-100 column and not recycled through CM-cellulose.

At each stage in the purification (see table 1) both the specific α-galactosidase and lectin activities were measured. Unlike the observations in [8], the lection/enzyme activity ratio did not remain the same during the purification. In stages I–III

Published by Elsevier Biomedical Press
00145793/82/0000—0000/$2.75

Table 1

Purification of α-galactosidase and the accompanying lectin activity from *Vicia faba* seeds[a]

Purification stage		Vol. (ml)	Enzyme act. (nkat/ml)	Protein (mg/ml)	(A) Spec. act. (nkat/mg)	(B) Lectin act. (HA/mg)	Ratio A/B
I. Crude extract		900	16	130.0	0.12	17600	7.0×10^{-6}
II. Citric acid precipitation		1000	15	30.0	0.50	20440	2.4×10^{-5}
III. $(NH_4)_2SO_4$ fractionation (25–65%)		95	117	130.0	0.90	12300	7.3×10^{-5}
IV. Sephadex G100 gel filtration	EI	90	57	4.0	14.2	1560	9.1×10^{-3}
	EII	75	48	1.4	34.3	2200	1.5×10^{-2}
V. CM-cellulose chromatography	EI	10	118	0.01	11800	650	18.1
	EII^1	2	50	0.41	122	7710	1.6×10^{-2}
	EII^2	4	210	0.18	1167	1800	0.65
VI. Melibiose–Sepharose	EI	3	250	0.02	12500	770	16.2
VII. α-Methyl mannoside–agarose	EI	1.2	456	0.04	11400	770	14.8

[a] 1 kg of seed powder was used

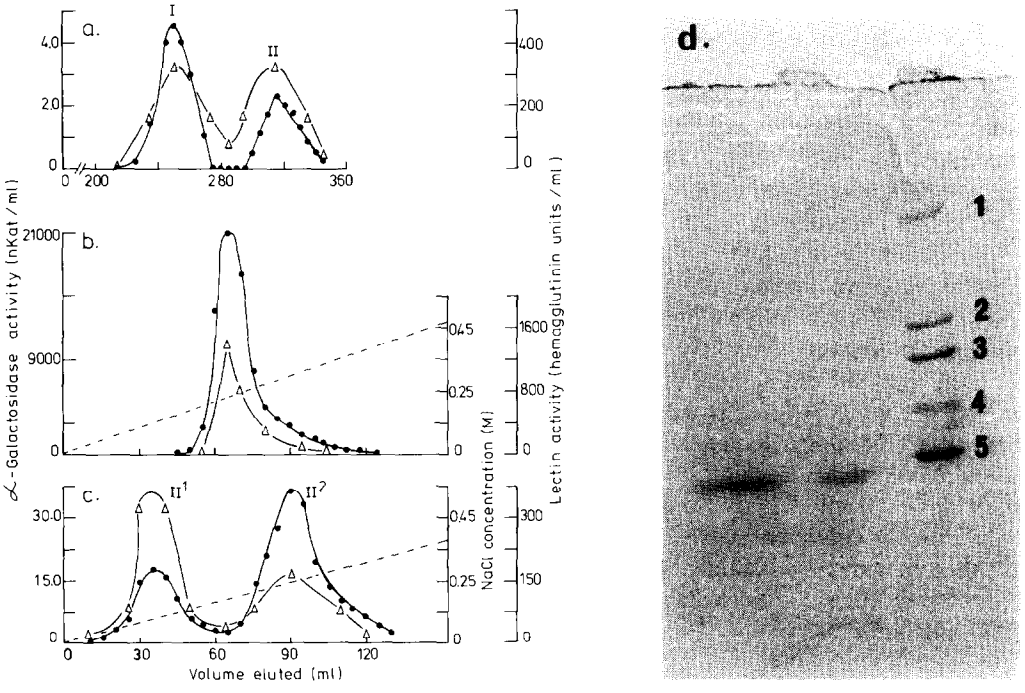

Fig. 1. (a) Sephadex G100 gel filtration profile of α-galactosidase from *V. faba* seeds. Enzyme preparation from stage III of table 1 was used; (b) CM-cellulose chromatography of α-galactosidase I; (c) CM-cellulose chromatography of α-galactosidase II; (●—●) α-galactosidase activity; (△—△) hemagglutinin activity; (– – –) NaCl gradient; (d) SDS–polyacrylamide slab gel electrophoresis of *V. faba* α-galactosidase I. The two wells to the left show enzyme samples (from stage V, table 1; 20 μg and 10 μg, respectively) stained with Coomassie blue. The following marker proteins (M_r $\times 10^{-3}$) were applied to the right well: 1, myosin (200000); 2, β-galactosidase (116000); 3, phosphorylase *b* (94000); 4, bovine serum albumin (68000); 5, ovalbumin (43000).

there was a 7-fold increase in enzyme specific activity whereas the lectin activity decreased. In the following stage (IV), lectin and enzyme (M_r 160000) activities co-eluted from a Sephadex G-100 column (fig. 1a) and there was a marked reduction in total lectin activity, presumably due to the removal of *Vicia faba* lectin (M_r 47500; [13]) and a further significant increase in enzyme purity. On passing enzyme I through CM-cellulose (fig. 1b; stage V) more lectin activity was lost, although the enzyme specific activity rose 800-fold in comparison with stage IV. (This enzyme preparation had a 10-fold higher specific activity than reported for enzyme I [10] although the amount of protein recovered was comparable. The loss of activity can presumably be explained by the greater number of purification stages used in [10].) Enzyme II from stage IV was resolved into two fractions, II^1 and II^2 on CM-cellulose (fig. 1c). Both of these fractions possessed higher specific agglutination activities than enzyme I. α-Galactosidase and lectin activities co-eluted in all cases in stage V.

When α-galactosidase I was examined by flat-bed SDS gel electrophoresis [15] it migrated as a single protein band (M_r 44000 ± 2000) which exhibited microheterogeneity. In comparison, the largest subunit of *V. faba* lectin has an M_r of 22500 [13].

To establish that none of the α-galactosidase fractions were contaminated with the *V. faba* lectin isolated in [13], which is devoid of α-galactosidase activity (N. Sumar, unpublished), all 3 purified forms were passed separately through a 3-*O*-methyl-D-glucosamine–CH-Sepharose affinity column [13] but no detectable α-galactosidase or lectin activity was absorbed. Furthermore, α-galactosidase I was retained entirely by an immobilized melibiose column (1.3 × 2.5 cm, 0.05 M acetate buffer, pH 4.0 at 4°C; Pierce and Warriner, UK) which is known to bind lectins and carbohydrate-binding proteins with α-D-galactose specificity [16]. On elution of this column with 10 mM PNPG and dialysis of the desorbed material the enzyme/haemagglutinin ratio was measured and shown to be essentially similar (stage VI, table 1) to that of the fraction applied to the column. Hence no contamination of enzyme I with the *V. faba* lectin was apparent. The binding of enzyme I to the melibiose affinity column could have occurred via catalytic and/or galactose-specific lectin sites on the enzyme. However, the presence of galactose-specific lectin sites appears unlikely as enzyme I was fully absorbed when passed through a methyl α-D-mannoside–agarose column (1.3 × 5 cm, 0.01 M phosphate buffer, pH 7.2 at 20°C; Sigma, London): this material is known to bind glucose/mannose-specific lectins. When the column was eluted with 0.5 M methyl α-D-mannoside and the resulting fraction dialysed, the enzyme/haemagglutinin ratio (stage VII, table 1) was similar to that observed at stage VI; the small difference is due to the loss of enzyme activity at stage VII brought about by the relatively high pH (7.2) used in this affinity step.

The lectin specificity of α-galactosidase I was further investigated by the hapten inhibition technique [17]. Table 2 shows the effects of various carbohydrates on rabbit erythrocyte agglutination. The data clearly show that D-mannose and to a lesser extent D-glucose and their low-M_r derivatives were significantly more effective in producing in-

Table 2

Sugar inhibition of hemagglutinin activity of α-galactosidase I from *Vicia faba* seeds

Sugar	Minimum concentration (mM) causing 50% inhibition of 6 hemagglutinin units [13]
D-Glucose	31.3
3-*O*-Methyl-D-glucopyranoside	31.3
Methyl-α-D-glucopyranoside	62.5
Methyl-β-D-glucopyranoside	250
N-Acetyl-glucosamine	31.3
D-Mannose	15.6
Methyl-α-D-mannopyranoside	3.1
D-Galactose	1000
Methyl-α-D-galactopyranoside	1000
D-Xylose	250
D-Arabinose	250
Melibiose	100
Stachyose	100
Raffinose	100
Sucrose	125
Galactomannan (clover)	0.125 mg/ml
Galactomannan (guar)	0.094 mg/ml
Glycogen	0.032 mg/ml
Starch (soluble)	0.003 mg/ml

hibition than D-galactose and its derivatives. Polysaccharides possessing glucosyl or mannosyl residues also acted as agglutination inhibitors.

α-Galactosidase I precipitated soluble starch and glycogen from solutions, and plots of turbidity against polysaccharide concentrations gave the normal characteristic bell-shaped curves [18,19]. Fig. 2 shows the interaction of yeast α-mannan with α-galactosidase I and the reciprocal relationship between the turbidity and the enzyme activity in the supernatant. Changes in the latter could be accounted for by changes in the activity in the precipitate. Methyl α-D-mannopyranoside at final conc. 50 mM prevented precipitation of the mannan in these experiments.

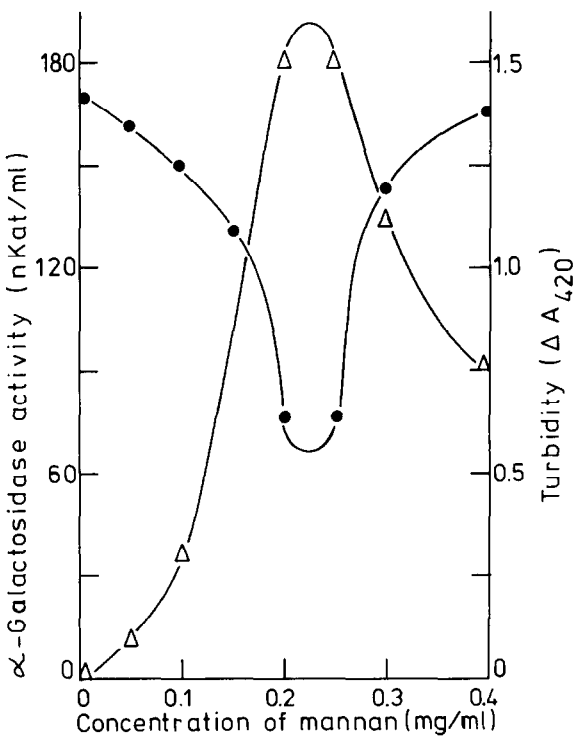

Fig. 2. Interaction of α-galactosidase I with yeast mannan. α-Galactosidase I (0.2 mg/ml) from stage V of table 1 was added to various concentrations of mannan solution in 0.1 M sodium acetate–HCl buffer (pH 6.1) and incubated at 25°C for 10 min. The turbidity formed was measured at 420 nm. The turbid solutions were centrifuged and the total activity in the supernatant and the precipitate was measured: (●—●) α-Galactosidase activity; (Δ—Δ) hemagglutinin activity.

In view of the close similarity between lectin and enzyme activities in *V. radiata* α-galactosidase reported in [8,20], a comparison of these activities was made with α-galactosidase I. Fig. 3 shows a

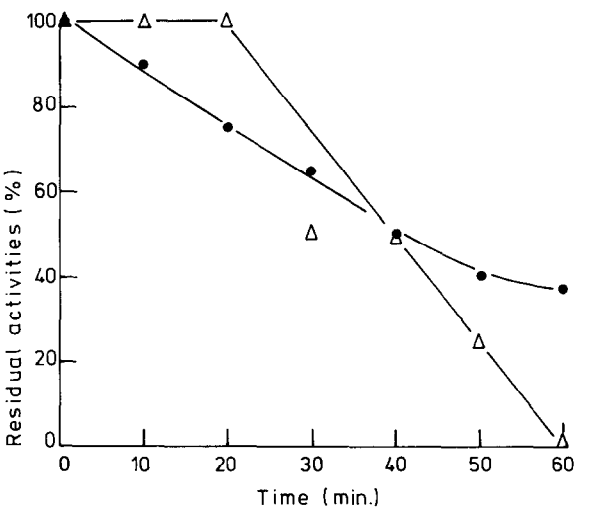

Fig. 3. Heat stability of α-galactosidase I. The enzyme (stage V of table 1) was incubated at 65°C in McIlvaine buffer (pH 5.5) for various time intervals and then the α-galactosidase (●—●) and hemagglutinin (Δ—Δ) activities were measured.

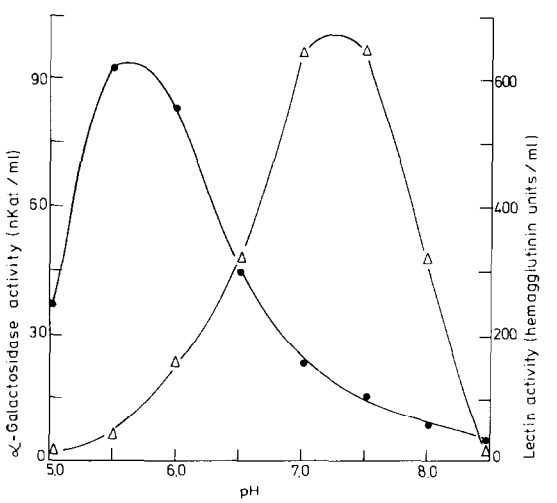

Fig. 4. pH optimum of hemagglutinin and enzyme activities of α-galactosidase I. Reactions were carried out in 0.1 M potassium phosphate buffer at various pH values using the enzyme from stage V of table 1; (●—●) α-galactosidase activity; (Δ—Δ) hemagglutinin activity.

difference in the heat stabilities of the lectin and catalytic activities of the *V. faba* enzyme. Fig. 4 shows that the optimum pH for agglutination is almost two units removed from that for the hydrolysis of PNPG.

A clear difference between the lectin and catalytic activities of α-galactosidase I can also be seen when the data in table 2 are compared with enzyme inhibition studies [21]. D-Galactose and galactose-containing oligosaccharides inhibited the enzyme activity but significantly higher concentrations of these compounds were needed to inhibit haemagglutination.

4. CONCLUSIONS

α-Galactosidase I possesses both enzymic and lectin activities and the latter is glucose/mannose specific. It seems very unlikely, in view of the purification procedure used, the affinity column studies and the molecular mass differences, that the enzyme preparation is composed of two proteins, an α-galactosidase and the *V. faba* lectin. All results suggest that the sites for both catalytic and lectin functions reside at separate loci on the same protein, and, hence, both in terms of lectin specificity and site separation, *V. faba* α-galactosidase I differs from the haemagglutinating α-galactosidase in [8]. In the latter case enzyme and lectin activities would appear to reside at identical or nearly identical sites. It is possible to explain the observations [8] in terms of a tetrameric α-galactosidase. Thus in the case of their agglutination studies the enzyme may first bind via its catalytic sites to terminal α-D-galactosyl residues on the surfaces of the red blood cells and then slowly hydrolyse, thereby causing the blood-clot to dissolve. It is also worth noting that the monomeric form of the enzyme from *Vigna radiata* [9] possessed no lectin activity, unlike the monomer II^2 of α-galactosidase I from *V. faba*. (Preliminary studies have shown that both II^1 and II^2 are also glucose/mannose-specific lectins.) In the case of the *Vigna* enzyme, this could result if monomer units possessed only single binding sites.

ACKNOWLEDGEMENTS

We are indebted to the Central Research Fund of the University of London for an equipment grant. S.N. received financial support from the British Council. Dr A.K. Allen (Department of Biochemistry, Charing Cross Hospital Medical School, London) provided us with a specimen of *Vicia faba* lectin and helpful comments on this study.

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